Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

Article

Aromatic Profiles of Essential Oil from 5 Commonly Used Thai Basils

Tibet Tangpao [1,2], Hsiao-Hang Chung[3], and Sarana Rose Sommano [2,*]

[1] Major of Biotechnology, the Graduate School of Chiang Mai University, Chiang Mai 50200, Thailand
[2] Plant Bioactive Compound Laboratory (BAC), Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[3] Department of Horticulture, National Ilan University, Yilan City, Yilan County 260, Taiwan (R.O.C.)
* Correspondence: sarana.s@cmu.ac.th

Abstract: The research objectives of this study are to analyse the volatile compositions of different basil types available in Thai markets and to descriptively determine their aromatic qualities. Essential oils were hydro-distillated from fresh leaves of 2 Holy basil (*Ocimum sanctum*) varieties namely, white and red and other basil species, including Tree basil (*O. gratissimum*), Sweet basil (*O. basilicum* var. thyrsiflorum) and Lemon basil (*O. citriodorum*). Oil physicochemical characteristics and volatile chromatograms from Gas Chromatography-Mass Spectrometry (GC-MS) were used to qualitatively and quantitatively describe the chemical compositions. Methyl eugenol, estragole and eugenol were among the major chemicals found in the essential oils of these basil types. Classification by Principal Component Analysis (PCA) advised that these *Ocimum* spp. samples are grouped based on either the distinctive anise, citrus aroma (estragole, geranial and neral) or spice-like aroma (β-methyl eugenol, caryophyllene and α-cubebene). The essential oil was also used for descriptive sensorial determination by five trained panelists, using the following developed terms: anisic, citrus, herb, spice, sweet and woody. The panelists were able to differentiate essential oil of white Holy basil from red Holy basil based on the intensity of the anisic attribute, while the anise and citrus scents were detected as dominant in the Lemon basil, Tree basil and Sweet basil essential oils. The overall benefit from this research was the elucidation of aromatic qualities from Thai common *Ocimum* species in order to assess their potential as the raw materials for future food research and development.

Keywords: *Ocimum* spp.; Essential Oil; Aromatic profiles; Thai food

1. Introduction

The genus of *Ocimum* (belonging to the Lamiaceae which also recognised as the richest essential oil bearing plant family) is represented by more than 150 species that are grown widely and distributed throughout the tropical and temperate regions [1]. They are collectively known as the "basils" which are in commercial demand for their nutritional, aromatic, ornamental, culinary, religious and medicinal importance [2]. Among those, Holy basil (*Ocimum sanctum*), Sweet basil (*O. basilicum*), Lemon basil (*O. citriodorum*) and Tree basil (*O. gratissimum*), are frequently cultivated in several countries of South and South-East Asia including Thailand as for culinary herbs [3,4]. Much recently demands are utmost for their essential oils not only for food but also for medicinal industries [1,5-7]. There has also been an increasing concern on liable health problems associated with synthetic food flavouring agent. Therefore, food researchers are focusing on the search of natural products that could replace the chemically synthetised food additive [8,9]. Essential oil as well as any other plant based natural ingredients have been the beneficiary of legal, regulatory and consumer preference as the result of a shared opinion on food safety [9,10].

Thai food plays an important part of Thai image in global eyes and is the most important reason for travelers to choose Thailand as their holiday destination [11,12]. Moreover, the use of genuine

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at Foods 2018, 7, 175; doi:10.3390/foods7110175

ingredients in Thai food is accepted globally as healthy food choice because of its nutritional value and most important factor supporting the authentic Thai flavour [13,14]. Thus, it has come as no surprise that food suppliers of ethnic food ingredients in quest for true Thai basils essential oil. However, the varieties of basil species available as raw material for essential oil extraction may leads to the confusion of true basils aroma. The research aim of this study is therefore to describe the aromatic identity of common Thai basils by chemical and sensory analyse as ultimately to set the ideal scent in natural food additive products.

2. Materials and Methods

2.1. Plant material

To obtain reliable source of raw material supply, the aerial parts of different stages were collected from 5 *Ocimum* spp. types viz., *O. sanctum* var. Rama (red), *O. sanctum* var. Shyama (white), *O. citriodorum*, *O. basilicum* var. thyrsiflorum, and *O. gratissimum* (100 plants/ type) at their flowering stages. Plants were grown at Mae-Hia Agricultural Training and Research Center, Division of Horticulture, Faculty of Agriculture, Chiang Mai University in an open-area (100 x 2,500 cm plot and the plant spacing was 50 cm^2) and maintained by watering 2 hr and 3 times per week using drip irrigation, and fertilizing [Urea (46-0-0) and NPK Fertiliser (15-15-15) in the ratio 1:3] once a month until flowering. After collections, plant specimens (leaf and floral specimens) were separated and their morphological appearances were recorded prior to sending them to the Department of Biology, Faculty of Science, Chiang Mai University for confirmation of their scientific and common names by the experts.

2.2. Essential oil extraction

At the laboratory, leaf tissues were parted from the stems and inflorescence. Fresh leaves (~300 g) were used for essential oil extraction in a 5 L Clevenger hydro-distillation apparatus containing 2.5 L of distilled-water and the extraction was for 2 hr at 150 °C. After allowing to cool down at room temperature, the essential oil was collected and treated with anhydrous sodium sulfate to remove the remaining water. The yields were averaged and calculated according to fresh weight of plant material [15].

2.3. Physical characteristics of essential oil

Colour of the essential oil was determined by physical observation in day light and under ultraviolet radiation using an ultra-violet chamber [16]. The essential oil from water distillation was dissolved in methanol (10% v/v) and scanned for the absorbance with the wavelengths ranging from 250 to 500 nm by the UV-visible spectrophotometer (SPECTROstar Nano; BMG LABTECH) [17].

2.4. Gas chromatography mass spectrometry (GC-MS)

The GC-MS analyses were accomplished with a Bruker-scion 436 GC-MS equipped with Rxi-5Sil MS columns (restek). Essential oil sample (2 µL at the dilution of 1%, v/v, in dichloromethane with a presence of 0.003 % w/v toluene as an internal standard) was injected in split mode (1:20). The oven temperature was set at 60 °C for 3 min, and increased by 2.5 °C/min until 240 °C where it was held at this temperature for 10 min. The carrier gas was Helium with a flow rate of 1.1 mL /min. The interface with MS was at 200 °C and mass spectra were taken at 70 eV in electron impact ionisation mode, with a scanning speed of 0.5 scans/s from m/z 20-350 [18]. The standard solution of C_8 - C_{20} n-alkane (Fluka® Analytical) in hexane was also used for the calculation of retention indices [19].

2.5. Descriptive analysis

Peer-reviewed version available at Foods 2018, 7, 175; doi:10.3390/foods7110175

Prior to sensory testing, 5 trained panelists (2 males, 3 females and age ranged from 25 – 37 years old) who had completed 3 days of orientation and a 12 h descriptive training course. Thereafter, they had developed attribute terms describing the odour of 5 essential oil on cotton ball (200 µL). Panelists tasted all samples and discussed the attribute definitions, attribute references, reference intensities, and evaluation procedures. Most references were consistent with those of the previous studies as described in Table 1, although some additional terms (anisic, herb and woody) were added.

At the day of testing, the essential oil (200 µL oil pipetted on clean and deodourised cotton ball) and reference attributes were presented in front of the same group of the panelists. They then evaluated the intensity of the given attributes in triplicates on the 15-point interval scale (0 = none, 15 = extra strong) [20]. Clean air was obtained between each assessment. A gap of 20 sec was sufficient to the individual odour assessments.

Table 1. Attributes and references used in evaluating in 5 essential oil odour

Odour attributes	Reference standard	n/15	Reference
Anisic	Anisic powder, 2 g	10/15	*
Citrus	Lemon extract (McCormick), 200 µL	8/15	[21]
Herb	Thyme (McCormick), 0.5 g	10/15	*
Spice	Ground allspice (McCormick), 0.5 g	8/15	[22]
Sweet	Vanilla flavour (McCormick), 200 µL	10/15	[23]
Woody	peanut peel 2 g with 100 µl DI water	7/15	*

*Added terms from the panelists

2.6. Statistical analysis

Differences of the descriptive analysis data was determined using Analysis Of Variance (ANOVA) using SPSS with a significance level of 0.05. Principal Component Analysis (PCA) was used to summarise graphical differences of volatile components of the essential oil and the descriptive data among the *Ocimum* spp. using XLSTAT ver. 2018.5.

3. Results and Discussion

2.1. Plant identification and physiochemical characteristics of the essential oil

Basils are widely distributed in tropical areas and are likely to have originated in South Asia (India). These herbaceous plants are of annual type, usually propagated through seeds [4]. The genus of *Ocimum* comprises of more than 65 species and is the biggest genera in Lamiaceae family worldwide [24]. Different basil species can be identified by morphological characterisations such as leaf shape and its colour, flower structures and colour, seed structure and characteristics (Table 2). However, due to extensive cultivation, inter and intra-specific cross hybridisation has occurred leading to polyploidy and different numbers of species, subspecies and varieties that are not significantly different in their appearances [24]. In our study, five *Ocimum* spp. types had their distinct morphological characteristics. The *O. sanctum* of white and red varieties (viz., Rama and Shyama) possesed different leaf colours. *O. citriodorum* and *O. basilicum* var. thyrsiflorum illustrated unique seed characteristic which was mucilaginous after soaking in water. *O. gratissimum* gave large leaf size about 45 cm^2 while *O. citriodorum* conferred leaf size around 3.5 cm^2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at Foods 2018, 7, 175; doi:10.3390/foods7110175

To obtain the essential oil, fresh basil leaves were extracted by hydro-distillation and the physicochemical characteristics were shown in Table 2. White Holy basil (*O. sanctum* var. Rama) and Sweet basil (*O. basilicum* var. thyrsiflorum) illustrated maximum yield ~ 0.4%, Lemon basil *(O. citriodorum)* and red Holy basil (*O. sanctum* var. Shyama) yielded the essential oil of ~ 0.33%. However, Tree basil *(O. gratissimum)* gave the least essential oil content (< ~ 0.2%). The colour (orange, yellow and colourless) of essential oil under day light were different from species to species (*O. gratissimum*, *O. citriodorum*, *O. sanctum* and *O. basilicum* var. thyrsiflorum) but not from variety to variety (*O. sanctum* var. Rama and Shyama). Variation of essential oil colours not only depends upon taxonomical characteristics but also rely on age of the plants as well as time of harvesting and different extraction techniques [25-27]. Siddique et al., [27] suggested that the chemical compositions of essential oil contribute largely on its colour. Moreover, the alteration the essential oil colour as a result of their compositions is suggested to be due to thermal degradation, oxidation, isomerisation, dehydrogenation and polymerisation [17,27-29].

Essential oil with different chemical compositions can absorb UV light at different wavelength therefore, illustrating variation of light reflection intensity [30,31]. In our experiment, white and red Holy basils essential oil reflected high UV light intensity, followed by moderate reflection intensity (Tree and Sweet basils) and low reflection intensity (Lemon basil) (Table 2). These were confirmed by absorbance spectrum patterns under various UV - visible wavelengths (220 - 500 nm) (Figure 1). The active chemical components of essential oil from *Ocimum* spp. plants are estragole (methyl chavicol), eugenol and methyl eugenol [32-34]. Dighe et al., [35] advised that UV spectrum of eugenol gave the maximum absorption at 220 – 230 nm and a smaller peak at 278 nm which are in accordant to our results. However, we found the tiny peaks at 269 – 275 instead of 278 nm (Figure 1).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

Table 2. Morphology of 5 *Ocimum* spp. and physicochemical characteristics of their essential oil

Characteristics	LB	RB	SB	TB	WB
			Plant morphology		
General	50–105 cm tall 	70–150 cm tall 	45–100 cm tall 	140–200 cm tall 	70–160 cm tall 
Leaf structure	leaf size ~ 3.5 x 1 cm, leaf elliptic – broadly obovate, glabrous except hairy midrib, veinlets and margin	leaf size ~ 4 x 1.5 cm, ovate-obovate, elliptic-oblong, surface patently hairy to clothed with soft spreading hair, Purple leaf	leaf size ~ 5.5 x 2 cm, leaf ovate-lanceolate to oblong-lanceolate, glabrous except hairy midrib, veinlets and margin	leaf size ~ 9 x 5 cm, leaf lanceolate, ovate or ovate-lanceolate, glabrous except hairy midrib	eaf size ~ 4 x 1.5 cm, leaf ovate-obovate, elliptic-oblong, surface patently hairy to clothed with soft spreading hair, green leaf
Inflorescence and floral structure	Inflorescence greenish, Flowers white, Calyx green, long hairy	Inflorescence purple, Flowers purplish, Calyx purple, patently hairy to densely pubescent	Inflorescence greenish, Flowers whitish pink, Calyx green, long hairy	Inflorescence greenish purple, Flowers yellowish white, Calyx greenish purple, hairy	Inflorescence green-greenish purple, Flowers purplish, Calyx green, patently hairy to densely pubescent

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

Seed characteristics	Seed brownish black, ellipsoid, mucilaginous	Seed brown, globose, non-mucilaginous	Seed brownish black, ellipsoid, mucilaginous	Seed brown, subglobose, non-mucilaginous	Seed brown, globose, non-mucilaginous
			Physicochemical Characteristics of essential oil		
Yield*	0.37 % ±0.12b	0.33 % ±0.06b	0.43 % ±0.09c	0.19 % ±0.05a	0.47 % ±0.09c
Colour under day light	Yellow	Clear	Yellow	Orange	Clear
Colour under UV light	+	+++	++	++	+++

+ = degree of UV light reflection intensity

*Values are mean ± SE (standard error) of 3 replications

LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama) ; SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil *(Ocimum sanctum* var. Rama)

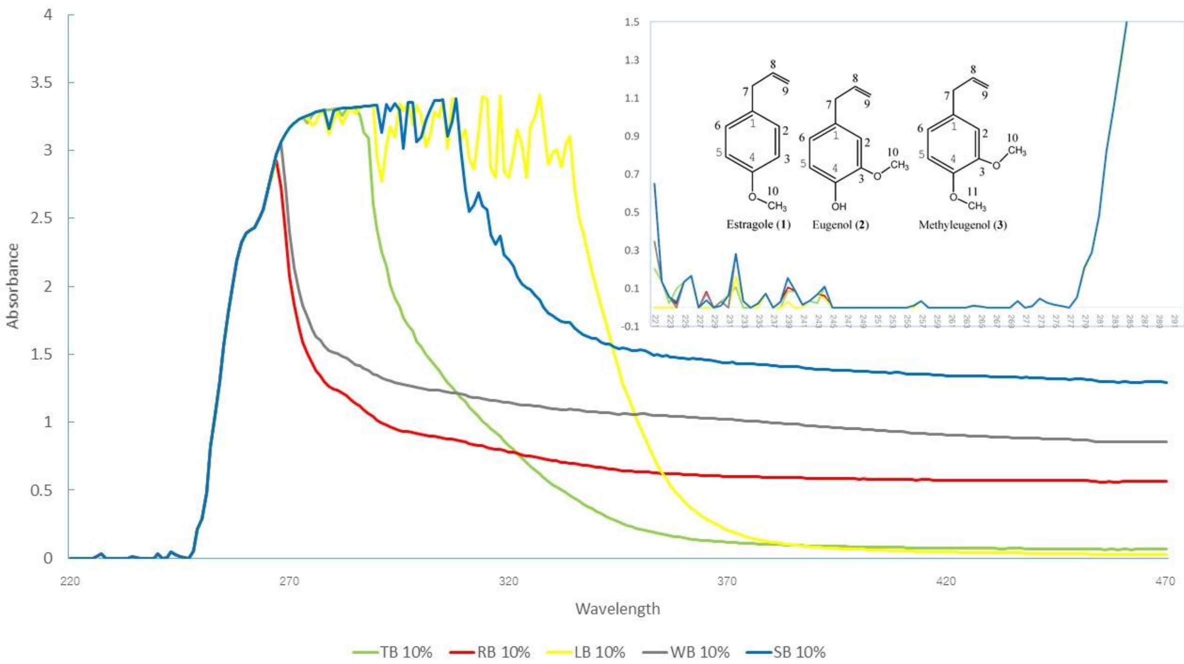

Figure 1. UV-visible spectra of the essential oils from 5 *Ocimum* spp. The essential oil was diluted in methanol. LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama); SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil (*Ocimum sanctum* var. Rama)**;** Chemical structures (1.) Estragole (2.) Eugenol (3.) Methyl Eugenol

2.2. Chemical profile of the essential oil

The concentrations and the calculated retention indices of volatile compounds from the 5 *Ocimum* spp. essential oil are exhibited in Table 3. Sixty-seven compounds were identified. The major components of essential oil from Lemon basil (*O. citriodorum*) volatile oil were estragole (methyl chavicol) (98.22 µg/ml), cittral (9.55 µg/mL) and neral (6.32 µg/mL). The principle components of Red Holy basil (*O. sanctum* var. Shyama) were methyl eugenol (683.90 µg/mL), β-Caryophyllene (145.81 µg/mL) and α-cubebene (104.70 µg/mL), and for Sweet basil (*O. basilicum* var. thyrsiflorum) were estragole (methyl chavicol) (452.80 µg/mL), geranial (180.57 µg/mL) and neral (151.16 µg/mL). The main compositions of Tree basil (*O. gratissimum*) were eugenol (408.00 µg/mL), α-ocimene (256.45 µg/mL) and γ-muurolene (91.57 µg/mL) and in oil of White Holy basil (*O. sanctum* var. Rama) essential oil were methyl eugenol (98.44 µg/mL), α-cubebene (9.94 µg/mL) and α-copaene (4.74 µg/mL).

From the literatures, three major compounds were generally identified from essential oil of *Ocimum* spp., viz. estragole (1), eugenol (2) and methyl eugenol (3) (Figure 1). Estragole (1) (methyl chavicol) is found in *O. basilicum* [36-39] and *O. citriodorum* [40]. Eugenol (2) is detected in *O. utricifolium* [40], *O. sanctum* var. green and var. purple [41], *O. gratissimum* [39,42,43] essential oil. Methyl eugenol (3) is the principal composition in *O. sanctrm* var Shyama [44], *O. uttricifolium* [45] and *O. campechianum* Mill. [46]. These results are in line with our work, however, the quantitative compositions of such chemicals may vary. Joshi and Sl [47] and Murarikova et al., [48] described that variation of the qualitative and quantitative of chemical profiles of the basil essential oil may be due to the variety of plant and their growing conditions such as season, climate or soil conditions.

The PCA between the volatile compositions and the essential oil types revealed three major clustering groups. The first group included Sweet basil and Lemon basil with the evident aromatic compounds (viz., estragole, geranial and neral) which represent anise, lime-like and fresh aroma [49]. The second cluster was of the white Holy basil and red Holy basil group with methyl eugenol, β-caryophyllene and α-cubebene (herb and spice) are distinctive compounds [50]. White and red Holy

Peer-reviewed version available at Foods 2018, 7, 175; doi:10.3390/foods7110175

basils are also variety related species (*O. sanctum*). The last group is the Tree basil that do not correlate with any groups described previously.

2.3. Sensory profile of the essential oil

Overall sensory scores obtained by the different 5 *Ocimum* spp. essential oils are illustrated in Table 4. The intensity (0 - 15) represent the maximum possible aroma quantity of each identified attributes judged by the panelists. It was observed that the sweet attribute (scores ~2.4 - 3.4) was not significantly different among all types of the essential oils. The essential oils of Tree basil and Sweet basil gave the highest intensity of herb odour (score ~ 9.0 - 11.0). Essential oils of white and red Holy basils provided the maximum woody scent (score ~2.2 - 3.4), while the Tree basil and white Holy basil dominated anisic attribute (score ~5.9 - 7.4). Tree basil and Sweet basil oils illustrated the highest spice aroma intensity (score ~6.4 - 7.3). The citrus scent was the highest in the oil of Lemon basil with the score of 12.8.

Table 3. Chemical composition of the essential oils from 5 *Ocimum* spp. plants

No.	Compounds	Retention index[b]	Amount of chemicals (µg/mL EOs)[a]				
			LB	RB	SB	TB	WB
1	Methyl 2-methylbutanoate	-	0	0	0	0.89	0
2	3-Hexen-1-ol	850.00	0	0	0	0.79	0
3	α-Pinene	930.13	0	0	0	0	0.32
4	Camphene	944.42	0	0	0	0	0.42
5	β-Pinene	973.25	1.31	0	2.58	0	0.52
6	1-Octen-3-ol	979.48	0	0	0	2.77	0
7	Myrcene	989.09	0	0	0	5.13	0
8	1,8-Cineole	1028.40	2.82	0	5.64	0	0
9	α-Ocimene	1049.30	0	0	12.99	256.45	0
10	Z-Ocimene	1051.41	5.83	0	0	17.66	0
11	γ-Terpinene	1058.22	0.36	0	2.39	0	0
12	3-Carene	1101.39	0	1.76	14.61	22.30	0
13	Linalool	1103.60	1.12	0	0	0	1.09
14	(4E,6Z)-allo-Ocimene	1132.13	0	0	0	14.90	0
15	d-camphor	1144.60	1.34	0	3.54	0	0
16	trans-Chrysanthemal	1151.52	0	0	2.20	0	0
17	Borneol	1167.87	0	7.79	0	0	2.80
18	1,4-Heptadiene, 3-methyl-	1168.98	0	0	5.54	0	0
19	Terpinen-4-ol	1180.06	1.78	0	15.38	0	0
20	Cyclohexane, ethenyl-	1189.20	0	0	6.78	0	0
21	Estragole	1210.93	98.22	0	452.80	0	0
22	(R)-α-pinene	1236.01	0	0	8.60	0	0
23	Neral	1251.13	6.32	0	151.16	0	0
24	(+)-(-)-3-carene	1263.67	0	0	6.30	0	0
25	Geranial	1282.32	0	0	180.57	0	0
26	Citral	1282.64	9.55	0	0	0	0
27	Eugenol	1370.61	0	0	0	408.00	1.50
28	α-Copaene	1379.21	0	20.72	0	16.09	4.74
29	β-Bourbonene	1387.81	0	7.68	0	1.88	0
30	4-Methylpyrazole	1389.25	0	0	1.82	0	0
31	N-Butylpyrrole	1391.40	0	0	0	0	2.25
32	β-Cubebene	1395.70	0	1.98	0	7.11	0
33	β-Elemene	1396.06	0.77	65.67	4.30	3.65	2.79
34	Methyl eugenol	1408.88	0.77	683.90	2.87	0	98.44

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

35	β-Caryophyllene	1423.55	1.34	145.81	10.60	17.27	0
36	α-Bergamotene	1439.00	1.00	0	4.49	0.69	0
37	(Z,E)-α-Farnesene	1441.70	0	0	0	51.61	0
38	α-Guaiene	1442.86	0.19	0	0	0	0
39	β-Sesquiphellandrene	1447.10	0	0	0	0.59	0
40	α-Humulene	1457.53	3.42	13.38	6.88	3.36	1.42
41	Bicyclo sesquiphellandrene	1467.95	2.43	0	10.79	0.59	0
42	Germacrene D	1469.11	2.26	0.55	14.33	0.89	1.23
43	γ-Muurolene	1489.58	0	0	0	91.57	0
44	α-Cubebene	1489.58	0	104.70	0	0	9.94
45	Bicyclo[3.1.1]hept-3-ene-spiro-2,4'-(1',3'-dioxane), 7,7-dimethyl-	1492.66	0	1.87	0	0	0
46	Bicyclogermacrene	1500.00	0.81	0	3.63	3.55	0
47	β-Gurjunene	1500.84	0	6.36	0	0	0
48	α-Selinene	1505.06	0	0	0	0	0.40
49	α-Bulnesene	1509.28	1.06	0	3.92	0	0
50	α-Farnesene	1510.97	0	0	0	42.33	0
51	α-Amorphene	1518.14	0	0	2.58	0	0
52	Phenylethanolamine	1518.99	0	0	0	0.69	0
53	δ-Cadinene	1524.89	0	8.12	0	11.05	0.56
54	1-Bromo-8-heptadecyne	1538.82	0.44	0	0	0	0
55	(Z)-4-Decen-1-ol	1539.24	0	3.84	0	0	0
56	(Z)-α-Bisabolene	1546.41	0	0	11.08	0	0
57	Eremophilene	1555.70	0	2.52	0	0	0
58	Elemol	1557.38	0	0	0	0	0.23
59	4-Ethylphenethylamine	1582.28	0	0	0	0.59	0
60	Ethyl trichloroacetate	1582.28	0	1.75	0	0	0
61	Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy-2,3-dihydro-2,2-dimethyl-	1589.87	0	0.99	0	0	0
62	1,3-diisopropyl-1,3-cyclopentadiene	1602.21	0	0	0	0.99	0
63	Cadina-1,4-diene	1621.68	0	0	1.24	0	0
64	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1622.12	0.31	0	0	0	0
65	Bromoacetonitrile	1649.56	0	0	0	0.50	0
66	α-Muurolene	1662.83	0	2.41	0	0	0
67	β-Bisabolene	1689.82	0.27	0	0	0	0

[a]Values are calculated as reference to internal standard toluene (0.003 % w/v)

[b] Retention index [33]

LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama); SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil (*Ocimum sanctum* var. Rama)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

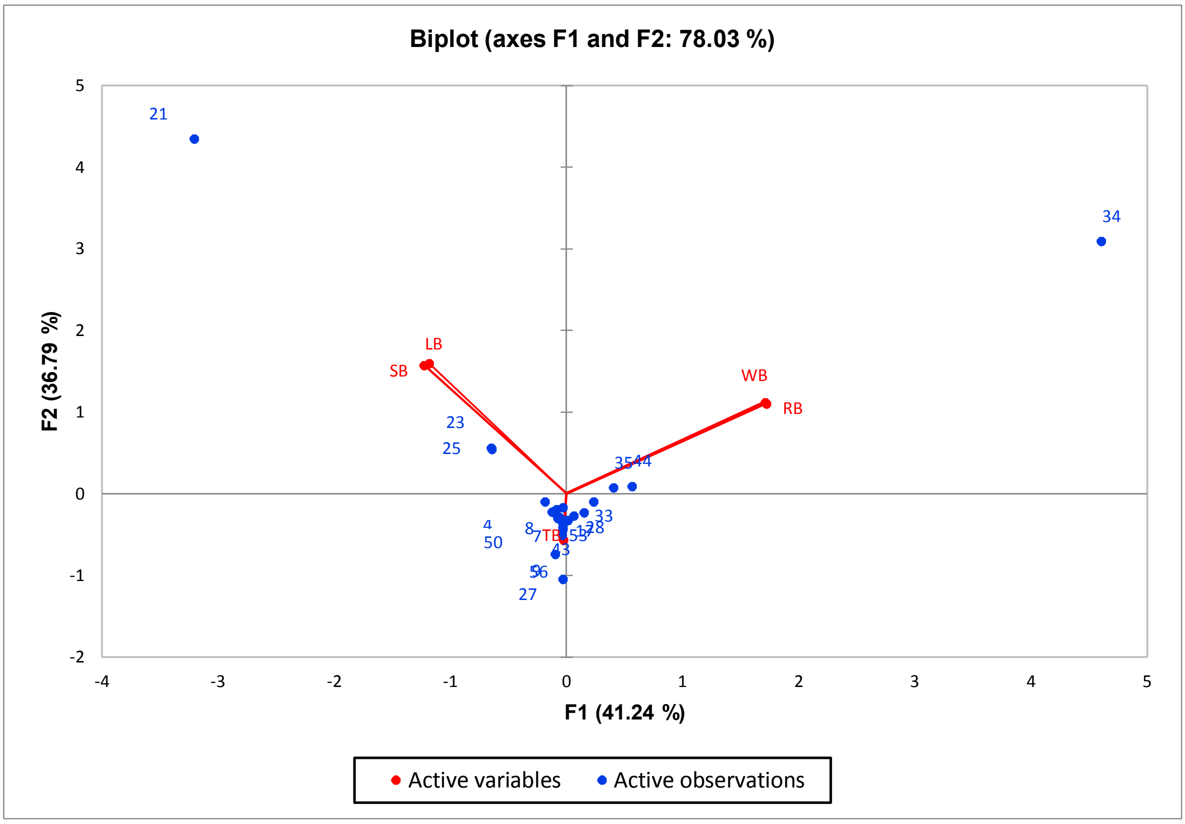

Figure 2. PCA biplot illustrating the relationships among the chemical components and 5 *Ocimum* spp. Odour active compounds of 1 – 64 correspond to the code compounds in Table 4. LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama); SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil (*Ocimum sanctum* var. Rama)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

Table 4. The mean intensity values of the six attributes for the five *Ocimum* spp. essential oil in descriptive sensory evaluation*

Basils	sweet	herb	woody	anisic	citrus	spice
LB	3.4 ±0.24**a**	7.2 ±0.49**bc**	0.98 ±0.02**a**	4.72 ±0.70**bc**	12.8 ±0.58**d**	5.2 ±0.66**b**
RB	2.6 ±0.51**a**	5.68 ±1.14**b**	2.26 ±0.55**bc**	3.6 ±0.51**ab**	2.7 ±0.37**a**	3.5 ±0.67**a**
SB	2.4 ±0.24**a**	11.2 ±0.86**d**	1.6 ±0.33**ab**	2.34 ±0.52**a**	10.62 ±0.69**c**	6.4 ±0.24**bc**
TB	2.46 ±0.63**a**	9.2 ±0.80**cd**	1.8 ±0.20**ab**	7.4 ±0.40**d**	8.9 ±0.56**b**	7.28 ±0.42**c**
WB	2.42 ±0.50**a**	1.1 ±0.10**a**	3.34 ±0.50**c**	5.96 ±0.87**cd**	4.2 ±0.37**a**	2.7 ±0.44**a**

LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama); SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil (*Ocimum sanctum* var. Rama)

* Mean scores (n= 5) for each attribute within a column with different letters are significantly different (P< 0.05) using Duncan's multiple comparison tests.

The PCA was able to split aromatic profile of white Holy basil relating with the higher intensity of anisic attribute. However, there was no correlation of woody and sweet attributes, with 5 essential oil types.

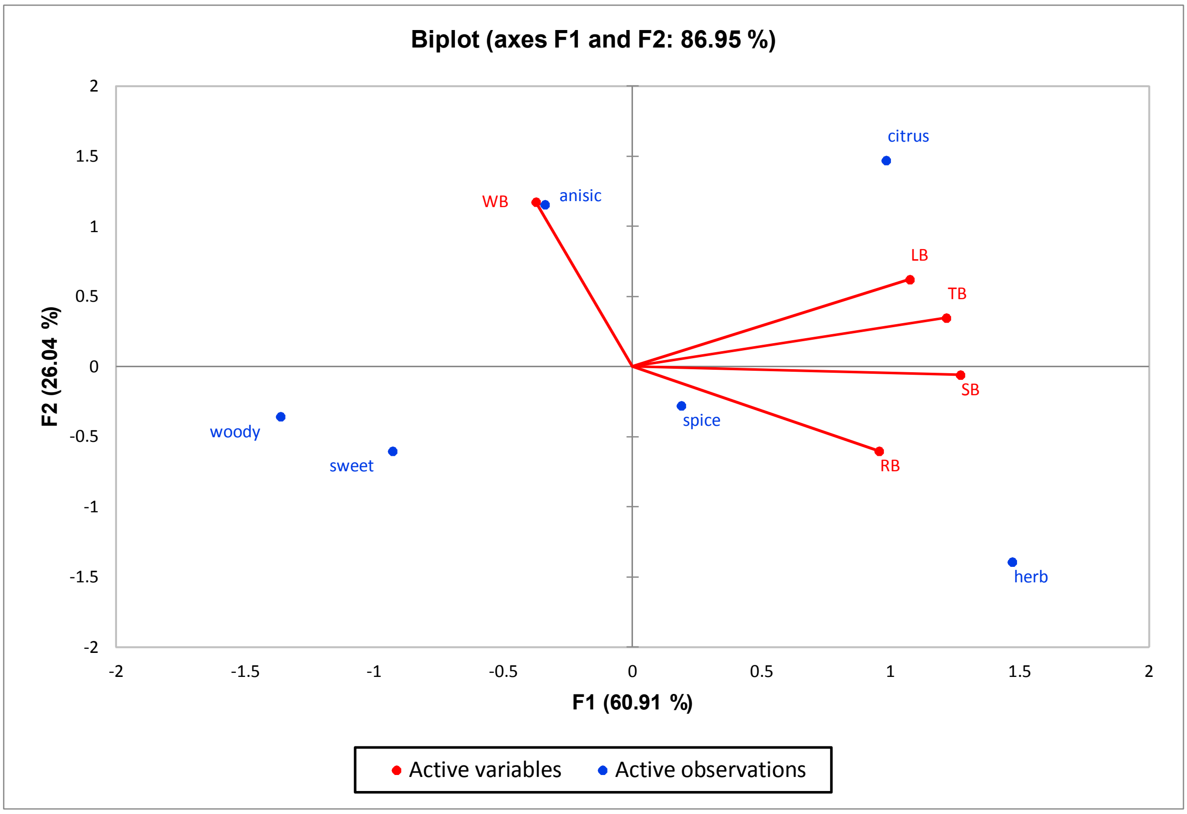

Figure 3. PCA biplot illustrating the relationships among the odour attributes and 5 *Ocimum* spp. LB = Lemon basil (*Ocimum citriodorum*); RB = red Holy basil (*Ocimum sanctum* var. Shyama); SB = Sweet basil (*Ocimum basilicum* var. thyrsiflorum); TB = Tree basil (*Ocimum gratissimum*); WB = white Holy basil (*Ocimum sanctum* var. Rama)

A research study on the attribute of essential oil from *Ocimum* spp. (*O. basilicum* L.) by Calín-Sánchez et al., [51] evaluated the same attributes that are herbaceous (herb), spice, woody and sweet. These attributes were also identified as in our study. This group of researchers also found that the Sweet basil (*O. basilicum*) essential oil extracted from the dried leaves gave stronger sweet and woody

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

attributes than the essential oil extracted from fresh leaves. In our study citrus attribute is a principle characteristic of essential oil from most Thai basil types analyses especially the Lemon basil as confirmed by both chemical and sensory evaluations. This is consistent with the previous research by Al-Kateb and Mottram [52] who studied the relationship between growth stages and volatile compositions of Lemon basil (*O. citriodorum* Vis.). They found that Citral, Linalool and Estragole major compounds of the essential oil and they contributed to the citrusy aroma.

4. Conclusions

From this research, we conclude that the major compositions of essential oil from the five types of basils used as food ingredient in Thailand are estragole, eugenol and methyl eugenol. From this chemical analysis we can then distinguish these *Ocimum* spp. plants from the odour characteristics into two groups: citrus and spice-like. Sensory analysis also confirm that citrus is the main feature in these *Ocimum* spp. essential oil. It is also possible to sensorially separate the aromatic scent of the two Holy basil (var. red and white) essential oils by using anisic attribute.

Funding: This research was funded by Thai Research Fund (TRF) under the Industrial-academic Research Fellowship program (IRF) and Handharvest, co. Ltd, Thailand.

Acknowledgments: We would like to thanks Dr. Paul Page for some contribution on this manuscript

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 September 2018 doi:10.20944/preprints201809.0338.v1

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

References

1. Pandey, A.K.; Singh, P.; Tripathi, N.N. Chemistry and bioactivities of essential oils of some *Ocimum* species: An overview. *Asian Pacific Journal of Tropical Biomedicine* **2014**, *4*, 682-694.
2. Patel, R.P.; Singh, R.; Rao, B.R.R.; Singh, R.R.; Srivastava, A.; Lal, R.K. Differential response of genotype×environment on phenology, essential oil yield and quality of natural aroma chemicals of five *Ocimum* species. *Industrial Crops and Products* **2016**, *87*, 210-217.
3. Juntachote, T.; Berghofer, E.; Siebenhandl, S.; Bauer, F. The antioxidative properties of holy basil and galangal in cooked ground pork. *Meat Science* **2006**, *72*, 446-456.
4. Mahajan, N.; Rawal, S.; Verma, M.; Poddar, M.; Alok, S. A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctum*. *Biomedicine & Preventive Nutrition* **2013**, *3*, 185-192.
5. Imen, T.; Olfa, B.; Jamel, H.; Luigi, C.P.; Mokhtar, L.; Guido, F.; Zeineb, O. Essential oil and volatile emissions of basil (*Ocimum basilicum*) leaves exposed to NaCl or Na_2SO_4 salinity. *Journal of Plant Nutrition and Soil Science* **2013**, *176*, 748-755.
6. Kwee, E.M.; Niemeyer, E.D. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. *Food Chemistry* **2011**, *128*, 1044-1050.
7. Jordán, M.J.; Quílez, M.; Luna, M.C.; Bekhradi, F.; Sotomayor, J.A.; Sánchez-Gómez, P.; Gil, M.I. Influence of water stress and storage time on preservation of the fresh volatile profile of three basil genotypes. *Food Chemistry* **2017**, *221*, 169-177.
8. Siripongvutikorn, S.; Thummaratwasik, P.; Huang, Y.-w. Antimicrobial and antioxidation effects of thai seasoning, tom-yum. *LWT - Food Science and Technology* **2005**, *38*, 347-352.
9. Wandersleben, T.; Morales, E.; Burgos-Díaz, C.; Barahona, T.; Labra, E.; Rubilar, M.; Salvo-Garrido, H. Enhancement of functional and nutritional properties of bread using a mix of natural ingredients from novel varieties of flaxseed and lupine. *LWT* **2018**, *91*, 48-54.
10. Burdock, G.A.; Wang, W. Our unrequited love for natural ingredients. *Food and Chemical Toxicology* **2017**, *107*, 37-46.
11. Poolklai, S. Food safety in thailand: A case of international senior tourists. *Procedia - Social and Behavioral Sciences* **2015**, *197*, 2120-2124.
12. Torres Chavarria, L.C.; Phakdee-auksorn, P. Understanding international tourists' attitudes towards street food in phuket, thailand. *Tourism Management Perspectives* **2017**, *21*, 66-73.
13. Youn, H.; Kim, J.-H. Effects of ingredients, names and stories about food origins on perceived authenticity and purchase intentions. *International Journal of Hospitality Management* **2017**, *63*, 11-21.
14. Piyavan, S.; B., B.J. Customer perceptions for expectations and acceptance of an authentic dining experience in thai restaurants. *Journal of Foodservice* **2007**, *18*, 69-75.
15. Jofré Barud, F.; López, S.; Tapia, A.; Feresin, G.E.; López, M.L. Attractant, sexual competitiveness enhancing and toxic activities of the essential oils from *Baccharis spartioides* and *Schinus polygama* on *Ceratitis capitata* wiedemann. *Industrial Crops and Products* **2014**, *62*, 299-304.
16. Barkatullah; Ibrar, M.; Rauf, A.; Inyat-Ur-Rahman. Physicochemical characterization of essential and fixed oils of *Skimmia laureola* and *Zanthoxylum armatum*. *Middle-East Journal of Medicinal Plants Research* **2012**, *1*, 51-58.
17. Taraj, K.; Delibashi, A.; Andoni, A.; Lazo, P.; Kokalari, E.; Lame, A.; Xhaxhiu, K.; Çomo, A. Extraction of chamomile essential oil by subcritical CO_2 and its analysis by uv-vis spectrophotometer. *Asian Journal of Chemistry* **2013**, *25*, 7361-7364.

Peer-reviewed version available at Foods 2018, 7, 175; doi:10.3390/foods7110175

18. De Lira, C.S.; Pontual, E.V.; de Albuquerque, L.P.; Paiva, L.M.; Paiva, P.M.G.; de Oliveira, J.V.; Napoleão, T.H.; Navarro, D.M.d.A.F. Evaluation of the toxicity of essential oil from *Alpinia purpurata* inflorescences to *Sitophilus zeamais* (maize weevil). *Crop Protection* **2015**, *71*, 95-100.
19. Adams, R.P. *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Allured Pub. Corporation: 2001.
20. Pimentel, T.C.; Gomes da Cruz, A.; Deliza, R. Sensory evaluation: sensory rating and scoring methods. In *Encyclopedia of food and health*, Caballero, B.; Finglas, P.M.; Toldrá, F., Eds. Academic Press: Oxford, 2016; pp 744-749.
21. Lee, J.; Chambers, D. A lexicon for flavor descriptive analysis of green tea. *Journal of Sensory Studies* **2007**, *22*, 256–272.
22. Ledeker, C.N.; Suwonsichon, S.; Chambers, D.H.; Adhikari, K. Comparison of sensory attributes in fresh mangoes and heat-treated mango purées prepared from thai cultivars. *LWT - Food Science and Technology* **2014**, *56*, 138-144.
23. Chambers, E.; Sanchez, K.; Phan, U.X.T.; Miller, R.; Civille, G.V.; Di Donfrancesco, B. Development of a "living" lexicon for descriptive sensory analysis of brewed coffee. *Journal of Sensory Studies* **2016**, *31*, 465-480.
24. Chowdhury, T.; Mandal, A.; Roy, S.C.; De Sarker, D. Diversity of the genus *Ocimum* (lamiaceae) through morpho-molecular (rapd) and chemical (GC–MS) analysis. *Journal of Genetic Engineering and Biotechnology* **2017**, *15*, 275-286.
25. Özcan, M.; Chalchat, J.-C. Essential oil composition of *Ocimum basilicum* L. And *Ocimum minimum* L. In turkey. *Turkey. Czech J. Food Sci.* **2002**, *20*, 223-228.
26. Sims, C.A.; Juliani, H.R.; Mentreddy, S.R.; Simon., J.E. Essential oils in holy basil (*Ocimum tenuiflorum* L.) as influenced by planting dates and harvest times in north alabama. *Journal of Medicinally Active Plants* **2014**, *2*, 33-41.
27. Siddique, A.B.; Mizanur Rahman, S.M.; Hossain, M.A. Chemical composition of essential oil by different extraction methods and fatty acid analysis of the leaves of stevia rebaudiana bertoni. *Arabian Journal of Chemistry* **2016**, *9*, S1185-S1189.
28. Turek, C.; Stintzing, F.C. Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety* **2013**, *12*, 40-53.
29. Hădărugă, D.I.; Hădărugă, N.G.; Costescu, C.I.; David, I.; Gruia, A.T. Thermal and oxidative stability of the *Ocimum basilicum* L. Essential oil/β-cyclodextrin supramolecular system. *Beilstein Journal of Organic Chemistry* **2014**, *10*, 2809-2820.
30. Beltramea, J.M.; Angnesa, R.A.; Chiavellib, L.U.R.; Costab, W.F.d.; Rosac, M.F.d.; Loboa, V.d.S.; Pominib, A.M. Photodegradation of essential oil from marjoram (*Origanum majorana* L.) studied by GC-MS and uv-vis spectroscopy. *La Revista latinoamericana de química* **2013**, *41*, 81-88.
31. Le Borgne, E.; Cicchetti, E.; Bertrand, T. HPTLC methods for qualitative and quantitative analysis of selected furocoumarins in essential oils. *Flavour and Fragrance Journal* **2017**, *32*, 330-339.
32. Simon, J.E.; Morales, M.R.; Phippen, W.B.; Vieira, R.F.; Hao, Z. Basil: A source of aroma compounds and a popular culinary and ornamental herb. In *Perspectives on new crops and new uses.*, Janick, J., Ed. ASHS Press: Alexandria, VA., 1999; pp 499-505.
33. Bernhardt, B.; Sipos, L.; Kókai, Z.; Gere, A.; Szabó, K.; Bernáth, J.; Sárosi, S. Comparison of different *Ocimum basilicum* L. gene bank accessions analyzed by GC–MS and sensory profile. *Industrial Crops and Products* **2015**, *67*, 498-508.

Peer-reviewed version available at *Foods* **2018**, *7*, 175; doi:10.3390/foods7110175

34. Singh, D.; Chaudhuri, P.K. A review on phytochemical and pharmacological properties of holy basil (*Ocimum sanctum* L.). *Industrial Crops and Products* **2018**, *118*, 367-382.
35. Dighe, V.V.; Gursale, A.A.; Sane, R.T.; Menon, S.; Patel, P.H. Quantitative determination of eugenol from *Cinnamomum tamala* nees and eberm. leaf powder and polyherbal formulation using reverse phase liquid chromatography. *Chromatographia* **2005**, *61*, 443-446.
36. Sajjadi, S.E. Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from iran. *Daru* **2006**, *14*, 128-130.
37. Mondawi, B.; Duprey, R.; Magboul, A.; Satti, A. Constituents of essential oil of *Ocimum basilicum* var. Thyrsiflorum. *Fitoterapia* **1984**, *55* 60-61.
38. Khatri, L.; Nasir, M.; Saleem, R.; Noor, F. Evaluation of pakistani sweet basil oil for commercial exploitation. *Pakistan Journal of Scientific and Industrial Research* **1995**, *38* 281-282.
39. Verma, R.S.; Bisht, P.S.; Padalia, R.C.; Saikia, D.; Chauhan, A. Chemical composition and antibacterial activity of essential oil from two *Ocimum* spp. grown in sub-tropical india during spring-summer cropping season. *Asian J Tradit Med* **2011**, *6*, 211-217.
40. Avetisyan, A.; Markosian, A.; Petrosyan, M.; Sahakyan, N.; Babayan, A.; Aloyan, S.; Trchounian, A. Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. *BMC Complement Altern Med* **2017**, *17*, 60.
41. Mondello, L.; Zappia, G.; Cotroneo, A.; Bonaccorsi, I.; Chowdhury, J.U.; Yusuf, M.; Dugo, G. Studies on the essential oil-bearing plants of bangladesh. Part viii. Composition of some *Ocimum* oils *O. Basilicum* L. Var. Purpurascens; *O. Sanctum* L. green; *O. Sanctum* L. purple; *O. americanum* L., citral type; *O. americanum* L., camphor type. *Flavour and Fragrance Journal* **2002**, *17*, 335-340.
42. Silva, M.G.V.; Matos, F.J.A.; Lopes, P.R.O.; Silva, F.O.; Holanda, M.T. Composition of essential oils from three *Ocimum* species obtained by steam and microwave distillation and supercritical CO_2 extraction. *ARKIVOC* **2004**, *6*, 66-71.
43. Sastry, K.P.; Kumar, R.R.; Arigar Kumar, N.; Sneha, G.; Elizabeth, M. Morpho-chemical description and antimicrobial activity of different *Ocimum* species. *Journal of Plant Development* **2012**, *19*, 53-64.
44. Awasthi, P.K.; Dixit, S.C. Chemical compositions of *Ocimum sanctum* shyama and *Ocimum sanctum* rama oils from the plains of northern india. *Journal of Essential Oil-Bearing Plants* **2007**, *10*, 292-296.
45. Janssen, A.M.; Scheffer, J.J.C.; Ntezurubanza, L.; Svendsen, A.B. Antimicrobial activities of some *Ocimum* species grown in rwanda. *Journal of Ethnopharmacology* **1989**, *26*, 57-63.
46. Zoghbi, M.D.G.B.; Oliveira, J.; Andrade, E.H.A.; Trigo, J.R.; Fonseca, R.C.M.; Rocha, A.E.S. Variation in volatiles of *Ocimum campechianum* mill. And *Ocimum gratissimum* L. Cultivated in the north of brazil. *Journal of Essential Oil-Bearing Plants* **2007**, *10*, 229-240.
47. Joshi, R.K.; Sl, H. Chemical composition of the essential oil of *Ocimum tenuiflorum* L. (krishna tulsi) from north west karnataka, india. *Plant Science Today* **2014**, *1(3)*, 99-102.
48. Murarikova, A.; Tazky, A.; Neugebauerova, J.; Plankova, A.; Jampilek, J.; Mucaji, P.; Mikus, P. Characterization of essential oil composition in different basil species and pot cultures by a GC-MS method. *Molecules* **2017**, *22*.
49. Lan Phi, N.T.; Minh Tu, N.T.; Nishiyama, C.; Sawamura, M. Characterisation of the odour volatiles in *Citrus aurantifolia* persa lime oil from vietnam. In *Developments in food science*, Bredie, W.L.P.; Petersen, M.A., Eds. Elsevier: 2006; Vol. 43, pp 193-196.
50. Xiao, Z.; Chen, J.; Niu, Y.; Chen, F. Characterization of the key odorants of fennel essential oils of different regions using GC–MS and GC–O combined with partial least squares regression. *Journal of Chromatography B* **2017**, *1063*, 226-234.

51. Calín-Sánchez, Á.; Lech, K.; Szumny, A.; Figiel, A.; Carbonell-Barrachina, Á.A. Volatile composition of sweet basil essential oil (*Ocimum basilicum* L.) as affected by drying method. *Food Research International* **2012**, *48*, 217-225.

52. Al-Kateb, H.; Mottram, D.S. The relationship between growth stages and aroma composition of lemon basil *Ocimum citriodorum* vis. *Food Chem* **2014**, *152*, 440-446.